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(71) Applicant: **KYOWA HAKKO KOGYO CO., LTD.**
6-1, Ohtemachi 1-chome
Chiyoda-ku Tokyo 100(JP)

(72) Inventor: **ISHINO, Shuichi**
1-12-2, Asahi-machi Machida-shi
Tokyo 194(JP)
Inventor: **HARA, Takahiro**
3-9-9, Naka-machi Machida-shi
Tokyo 194(JP)
Inventor: **TESHIBA, Sadao**
1626-9, Nogaya-cho Machida-shi
Tokyo 194-01(JP)

(74) Representative: **Casalonga, Axel et al**
BUREAU D.A. CASALONGA - JOSSE
Morassistrasse 8
W-8000 München 5(DE)

(54) ARGININE-RICH YEAST.

(57) An ethionine-resistant arginine-rich yeast belonging to the species Kluyveromyces polysporus.

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Technical Field

The present invention relates to yeast capable of accumulating L-arginine in the cells in large quantities. The yeast cells of the present invention are expected to be utilized as feed for livestock.

Background Art

Heretofore yeast cells such as Torula yeast and beer yeast have been utilized as feed for livestock.

However, some livestock cannot be sufficiently supplied with some amino acids from yeast cell source alone. For example, domestic fowls tend to suffer from insufficiency of L-arginine. Insufficient amino acids are supplemented by addition of each amino acid, but this is not economically advantageous. If various kinds of yeasts are provided which are capable of accumulating insufficient amino acids in the cells in large quantities, the yeast cells would be widely utilized as feed for livestock.

As yeast capable of accumulating L-arginine in the cells in large quantities, there is known a regulatory mutant (argR, cpoO) belonging to the genus Saccharomyces which accumulates 1.7% of L-arginine in the cells [Eur. J. Biochem., 12, No. 1, 40-47 (1970)].

Disclosure of the Invention

According to the present invention, ethionine-resistant strains belonging to the genus Kluyveromyces can be provided as yeast capable of accumulating L-arginine in the cells in large quantities. These strains are yeasts with a high arginine content which contain at least 5% (on a dry cell weight basis) of free L-arginine in the cells.

The yeast with a high arginine content of the present invention includes any yeast which belongs to Kluyveromyces polysporus and has resistance to ethionine. Yeast having such properties can be obtained by using yeast belonging to the genus Kluyveromyces as a parent strain, and subjecting the strain to a conventional mutation treatment, for example, UV irradiation or a chemical treatment with N-methyl-N'-nitro-N-nitrosoguanidine, etc. and selecting yeast capable of accumulating at least 5% (on a dry cell weight basis) of L-arginine in the cells from mutants which have acquired resistance to ethionine. Any yeast may be usable as the parent strain so long as it belongs to the genus Kluyveromyces. A specific example is Kluyveromyces polysporus IFO 0996 [Int. J. Syst. Bact., 38, 822 (1983)].

The process for obtaining the yeast with a high arginine content of the present invention is described specifically below.

As the parent strain, Kluyveromyces polysporus IFO 0996 was used. Cells of the parent strain were suspended in a physiological saline solution to a density of 10^8 cells/mℓ. The suspension (0.1 mℓ) was smeared on a minimum agar medium [20 g/ℓ glucose, 6.7 g/ℓ Yeast Nitrogen Base (manufactured by Difco), 20 g/ℓ agar; pH 5-6] containing 1000 μg/mℓ ethionine. The plate was put under a UV light source (15W ultraviolet lamp, wavelength; 253Å) at a distance of 35 cm from the light source and exposed to UV rays for 20 seconds. The agar plate irradiated with UV rays was incubated at 30°C for 2-4 days. About 250 colonies of ethionine-resistant strains grew on the surface of the ethionine-containing medium. Two hundred ethionine-resistant strains were picked up and cultured in a manner similar to Example 1 described below. Some ten strains which accumulated more than 5.0% of L-arginine in the cells were selected. One of them was named Kluyveromyces polysporus ETA82-33 and was deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan as FERM BP-2560 on August 22, 1989, under the Budapest Treaty.

As the medium for culturing the yeast with a high arginine content of the present invention, there may be used a nutrient medium or a synthetic medium containing carbon sources, nitrogen sources, inorganic salts, growth factors, etc.

As the carbon source, any carbohydrates such as glucose, fructose, sucrose, molasses, starch, starch hydrolyzate and fruit juice, alcohols such as ethanol, methanol and propanol, etc. may be used so long as the yeast can assimilate them.

As the nitrogen source, ammonium sulfate, ammonium nitrate, ammonium chloride, ammonium phosphate, ammonium acetate, urea, ammonia, amines, peptone, meat extract, yeast extract, corn steep liquor, casein hydrolyzate, various fermented cells and their digestion products, etc. may be used.

As the inorganic salts, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, magnesium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, calcium carbonate, etc. may be used.

When nutrient-requiring mutants are used, preparations of nutrients which are growth factors or natural substances containing the nutrients are added. For example, amino acids such as lysine and glutamic acid, vitamins such as biotin and thiamine, and nucleic acid bases such as purine and adenine are used.

Cultivation is carried out under aerobic conditions, for example, with aeration and agitation, by maintaining the temperature at 24 - 37°C and the pH at 4-9, and is completed generally in 2 to 7

days. The pH is controlled by using urea, calcium carbonate, ammonia gas, aqueous ammonia, magnesium phosphate, ammonium carbonate, or the like. After the completion of cultivation, the cells are harvested from the culture and dried, whereby the yeast with a high arginine content can be obtained. The cells can be harvested and dried by known techniques, e.g., by using a sharples and a spray drier.

Best Mode for Carrying Out the Invention

Example 1

Kluyveromyces polysporus ETA82-33 was used as the seed strain. ETA 82-33 was inoculated into a test tube containing 5 ml of a seed medium comprising 10 g/l glucose, 5 g/l peptone and 3 g/l yeast extract (pH 6.0), and subjected to shaking culture at 30°C for 24 hours.

The resulting seed culture (0.05 ml) was inoculated into a test tube containing 6 ml of a cell culture medium [50 g/l glucose, 34 g/l corn steep liquor, 3.8 g/l $(NH_4)_2SO_4$, 1 g/l $MgSO_4 \cdot 7H_2O$, 1 g/l KH_2PO_4, 30 g/l $CaCO_3$ (pH 6.5)]. Cultivation was carried out with shaking at 30°C for 48 hours. After the completion of cultivation, the cells were harvested by centrifugation and washed twice with 67 mM phosphate buffer (pH 6.0). Then, 0.1 g (on a dry cell weight basis) of the yeast cells was suspended in 2 ml of 67 mM phosphate buffer, followed by extraction at 100°C for 10 minutes. Free L-arginine in the extract was quantitatively determined and it was found that 8.0% of L-arginine was accumulated based on the dry cell weight.

As a control, Kluyveromyces polysporus IFO 0996, which is the parent strain, was cultured in the same manner as described above. The amount of L-arginine accumulated was 0.2% based on the dry cell weight.

Example 2

Kluyveromyces polysporus ETA82-33 was used as the seed strain. ETA 82-33 was inoculated into a test tube containing 30 ml of a seed medium comprising 10 g/l glucose, 5 g/l peptone and 3 g/l yeast extract (pH 6.0), and subjected to shaking culture at 30°C for 24 hours.

The whole of the resulting seed culture was inoculated into a 2-l fermenter containing 1000 ml of a cell culture medium [50 g/l glucose, 34 g/l corn steep liquor, 3.8 g/l $(NH_4)_2SO_4$, 1 g/l $MgSO_4 \cdot 7H_2O$, 1 g/l KH_2PO_4, 30 g/l $CaCO_3$ (pH 6.5)]. Cultivation was carried out with stirring at 30°C for 40 hours, during which the pH was adjusted to 5.5 with aqueous ammonia. After the completion of cultivation, the cells were harvested by centrifugation and washed twice with 67 mM phosphate buffer (pH 6.0). Then, 0.1 g (on a dry cell weight basis) of the yeast cells was suspended in 2 ml of 67 mM phosphate buffer, followed by extraction at 100°C for 10 minutes. Free L-arginine in the extract was quantitatively determined and it was found that 13.0% of L-arginine was accumulated based on the dry cell weight.

As a control, Kluyveromyces polysporus IFO 0996, which is the parent strain, was cultured in the same manner as described above. The amount of L-arginine accumulated was 0.2% based on the dry cell weight.

Industrial Applicability

According to the present invention, there can be provided yeast with a high L-arginine content which is useful as yeast for feed.

Claims

1. A yeast belonging to Kluyveromyces polysporus and having resistance to ethionine.

2. The yeast according to claim 1, wherein said yeast is a yeast with a high arginine content which contains at least 5% (on a dry cell weight basis) of free L-arginine in the cells.

3. The yeast according to claim 1 or 2, wherein said yeast is Kluyveromyces polysporus ETA 82-33 (FERM BP-2560).

4. A process for producing a yeast with a high arginine content, which comprises culturing in a medium a yeast belonging to the genus Kluyveromyces and having resistance to ethionine, allowing a yeast containing at least 5% (on a dry cell weight basis) of free L-arginine in the cells to accumulate in the culture, and recovering said yeast therefrom.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP90/01080

I. CLASSIFICATI N OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl[5] C12N1/16//(C12N1/16, C12R1:645)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C12N1/16, C12P13/10

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

BIOSIS DATA BASE

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	JP, B2, 56-46826 (Kojin Ltd.), 5 November 1981 (05. 11. 81), (Family: none)	1 - 4
A	J. Bacteriology, Vol.142, No.2 (May, 1980), American Society for Microbiology, JAMES V. BRAWLEY and ADOLPH J. FERRO "Stimulation of Yeast Ascospore Germination and Outgrowth by S-Adeno-sylmethionine" p.608-p.614	1 - 4
A	Eur. J. Biochem. 12 No.1 (1970), F. RAMOS, P. THURIAUX, J. M. WIAME, and J. BECHET "The Porticipation of Ornithine and Citrulline in the Regulation of Arginine Metabolism in Saccharomyces cerevisiae" p.40-p.47	1 - 4

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
October 12, 1990 (12. 10. 90)	October 29, 1990 (29. 10. 90)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	

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